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EXPERIMENTAL ERYSIPELAS

STUDIES IN STREPTOCOCCUS INFECTION AND IMMUNITY. IV *

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Erysipelas was probably the first recognized of those human diseases which we now know to be due to the streptococcus. Its striking clinical appearance made it recognizable as an entity even in ancient times. Erysipelas is a disease of much interest as the first of the streptococcus infections from which the causative organism was isolated. It is a disease the instances of which are apparently diminishing, owing largely to the introduction of aseptic and antiseptic treatment of wounds.

The essential facts concerning clinical erysipelas may be briefly outlined as follows:[1]

The forms of erysipelas have at times been classified as idiopathic and traumatic. From the present conception, however, this point of differentiation is scarcely valid, since trauma must be present even in the so-called idiopathic forms. Erysipelas tends to occur in families, and is present more frequently in adults and in old age. There are said to be more cases among females than among males, although it is more fatal in the latter sex. The occurrence of erysipelas in children, though not so notable, is marked by great severity. The mortality in general is given as from 3-10%.

Erysipelas occurs most frequently on the face or back, and in infants about the umbilicus. The inoculation, which occurs through slight abrasion or as a spreading process from some previously infected wound, is followed by a short period of incubation, of from 15 to 60 hours. The characteristic lesion of erysipelas is an infection of the superficial layers of skin and spreads by extension. In general it takes the form of a sharply demarcated, red, hot, indurated area, which spreads rather rapidly with a characteristically raised, glistening margin, leaving behind, as the process extends, a less inflamed yellowish or

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Gay, F. P., and Stone, R. L.: Jour. Infect. Dis., 1920, 26, p. 265.
Gay, F. P., and Morrison, L. F.: Ibid, 1921, 28, p. 1.
Gay, F. P., and Rhodes, B.: Ibid., 29, p. 217.
Of correlative interest are the following articles by our associates:
Cook, M. W.; Mix, V., and Culvyhouse, E. O.: Ibid., 1921, 28, p. 93.
Foster, L. F., and Randall, S. B.: Jour. Bacteriol., 1921, 6, p. 143.
Foster, L. F.: Ibid., p. 161.
Foster, L F.: Ibid., p. 211.

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[1] Lambert, A. V. S.: Nelson System of Med., 1920, 1, p. 446. Tileston, W.: Oxford Med., 1921, 4, p. 887. Anders, J. M.: Osler's Modern Med., 1907, 2, p. 525. Kolle, W., and Hetsch, H.: Die exper. Bakteriol. und die Infektionskrankheiten, 1911, 1, p. 397. Jochmann: Lehrbuch der inf. Krankheiten, 1914, p. 521. Ker, C. B.: Oxford Pub., 1920, p. 465. Sörensen, E.: Ztschr. f. Hyg. u. Infektionskr., 1908, 62, p. 363.

brownish center. This spreading infection is accompanied by a fever of considerably intensity, to 104 F. or even higher, which is continuous or remittent in form and usually ends by crisis. Erysipelas is not ordinarily a painful infection, but it is accompanied by a sensation of burning. Blebs occur frequently on the surface; suppuration, though often present, is not a characteristic feature.

A more minute examination of the lesion itself shows that the streptococcus, which is the cause of the disease, is found in greatest numbers in the advancing indurated margin. The organisms are seen filling the lymphatic spaces of the corium, which explains the method of the spread of the infection. The advancing margin of infection is not characterized by the occurrence of any considerable number of polymorphonuclear leukocytes, as is usual in the more pyogenic types of streptococcus infection, but by the presence of mononuclear cells (MacCallum[2]). According to Unna,[3] the streptococcus of erysipelas is negatively chemotactic for leukocytes that bear the oxygen necessary for its growth. It, therefore, must seek those portions of the skin in the lymphatic spaces and connective tissue that contain most oxygen, which explains its characteristic localization. Dead streptococci, however, fall prey to the polymorphonuclears, which accounts for the accumulation of these cells in the older regions of the process and the formation of a certain amount, though never of a predominating quantity, of pus. The former contention of Fehleisen[4] that true erysipelas must by definition be a nonsuppurative process would, we believe, be no longer tenable.

Erysipelas is usually self-limiting and does not ordinarily produce complications in the form of pyogenic foci or septicemia. The process lasts in uncomplicated instances for from 4 to 14 days.

Relapses or exacerbations of erysipelas are said to occur in about 10% of the cases, that is to say, relatively frequently. These relapses should be differentiated from the recurrences of the process, which have been regarded as one of its most characteristic features. It has been recognized that there exists a wide individual variation in susceptibility to erysipelas, as is evidenced not only by the nonoccurrence of the process in most persons, but particularly by the repeated occurrence in certain hypersusceptible persons. Various estimates have shown that adults suffering from erysipelas give a history of previous attacks in from 9 (Anders) to 20% (Tileston) of cases. As many as 40 attacks have been reported in a single person. This tendency to recurrence of the disease is one of the arguments that has been used to prove the nonexistence of any permanent active immunity against streptococcus infections. There are, however, other data, particularly of an experimental nature, in man as well as in animals, which seem to show equally clearly that recovery from one attack is followed by at least a transitory protection. It is evident that some form of increased resistance must occur or there would be no self-limitation of the process (Kolle and Hetsch[1]). That recovery is not due to the dying out of the streptococci, but to a real though temporary increased resistance, is evidenced by the fact that the organisms have been found for considerable periods after recovery in the scales of the subsiding lesion (Nobel and Zilczer[5]). This is further indicated in the observations of Levaditi,[6] who found that healing streptococcus wounds have the property of destroying the particular streptococci that had been present in them. This matter of temporary, and perhaps local, acquired immunity is one that interests us primarily in this article and can be more profitably discussed in connection with the experimental production of erysipelas in rabbits.

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[2] Text Book of Pathology, 1920, p. 521.

[3] Deutsch. med. Wchnschr., 1921, 47, p. 1349.

[4] Fehleisen, F., 1883.

[5] Med. Klin., 1918, 14, p. 491.

[6] Compt. rend. Soc. Biol., 1918, 81, p. 1059.

Experimental erysipelas was probably first produced by Orth[7] in 1873, who, before the knowledge of the causative organism in this disease, produced a spreading and fatal abscess by subcutaneous inoculation on the back of rabbits of the contents from a human erysipelatous bleb. It is quite possible also that he actually succeeded in growing pure cultures of the "microspheres," which he found associated with both the natural and experimental lesion. In 1881 Fehleisen[4] began his studies on the histologic findings in cases of erysipelas, and in 1883 published his completed work, which included the growth in pure culture of the streptococcus constantly present in this lesion, to which he gave the name of Streptococcus erysipelatis. This streptococcus was not only present in the lymph spaces of the corium, particularly about the edges of the lesion, but after growth in blood serum and gelatine was capable of reproducing the disease when inoculated subcutaneously or by scarification on the ear in 9 rabbits. An incubation period of 3 or 4 days was followed by a rise in temperature, a sharply defined reddening, which extended to the root of the ear or over the head and neck. Fehleisen also reproduced the disease with this culture in several human beings suffering from malignant tumors, in some of whom a temporary retrogression of the tumor followed. There was likewise some evidence in the work of Fehleisen, as he himself appreciates from the study of these human cases, that a transitory immunity to reinfection with erysipelas exists, since second inoculation in certain of the originally positive cases resulted in failure.

The immediate sequel to this epoch-making study of Fehleisen was a discussion as to the specificity of the particular organism which he and subsequently others were able to isolate from this disease. In the following year, 1884, Rosenbach[8] isolated a streptococcus from purulent processes, to which he gave the name of Streptococcus pyogenes. Although at first an attempt was made to differentiate between these two varieties of streptococcus, it soon became evident that it could not be done, at least on the basis of their individual pathogenic effect on animals. A fundamental misconception as to comparative pathogenicity became firmly imbedded in the minds of the bacteriologists of this era in their efforts to fulfill too literally that particular postulate of Koch which prescribes that an organism to be proved to be the cause of any given disease in man, must, after isolation in pure culture, be capable of reproducing the same disease in a laboratory animal.✓We now know that such reproduction of disease is often impossible, particularly in the most characteristically human maladies. Attempts are still made to judge the original pathogenic significance of organisms isolated from human beings by measuring their lethal effects in animals. It is characteristically true, however, that the streptococcus usually shows the directly opposite results when the pathogenic effects of a given strain are measured in man and in rabbits; human pathogenic strains are relatively nonpathogenic for rabbits, and the reverse is often the case. At all events, in the case of erysipelas, although it so happens that a reproduction of the human disease in rabbits is possible, this fact by no means serves to prove that the organism originally isolated from human cases is capable of producing that disease and no other, either in man or animals. It was soon shown by Biondi,[9] Guarineri,[10] Petruschky[11] and many others that streptococci isolated from septicemia and various pyogenic processes in man will all produce erysipelas in the rabbit ear equally well. It was further shown that erysipelas is not wholly etiologically specific in man. From the early work of Bonome[12] and others it has been shown that other bacteria, such as staphylococci and colon bacilli and what are now recognized to be other varieties of streptococcus, may also produce erysipelas. It should further have been evident that erysipelas is not produced by a streptococcus endowed with some specific action when we consider that the disease occurs following and by direct infection from streptococcus wounds, and conversely that erysipelas may be followed by complications in the nature of septicemia and localized abscesses. The idea of a specific localizing affinity for any given organism has, however, by no means been abandoned, as is evidenced by what is generally regarded as a misinterpretation of experimental possibilities in the recent work of Rosenow.[13]

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[7] Arch. f. Exper. Path., 1873, 1, p. 81.

[8] Wiesbaden, 1884.

[9] Deutsch. med. Wchnschr., 1886, N.S. p. 132.

[10] Baumgartens Jahresbericht, 1887, 3, p. 32.

[11] Ztschr. f. Hyg. u. Infektionskr., 1896, 23, p. 142.

Having determined, then, that the streptococcus from erysipelas is from the standpoint of pathogenicity not endowed with unique and unchangeable specificity, it became evident from the subsequent experimental work in rabbits that these erysipelas strains differ in the results they produce in accordance with their particular and temporary pathogenicity for rabbits. Most of the observers, for example, who immediately followed Fehleisen,[4] notably Hajeck,[14] Denuce,[15] and Metchnikoff,[16] produced more discrete and purulent lesions with the strains of the cocci that they had isolated. In other words, they were simply dealing with strains less pathogenic for rabbits. In other hands streptococcus cultures became so pathogenic after passage through animals as to produce septicemia and death instead of erypipelas, unless minimal doses were employed.

With the gradual differentiation of streptococci from various sources by means of their biologic reactions, it soon became clear that the streptococcus which is found in nearly every case of erysipelas belongs to the group we now classify as Streptococcus pyogenes in its modern acceptance as presented by Holman;[17] that is to say, it is a hemolytic variety, which ferments lactose and salicin but not mannite. That this is the variety found almost without exception in erysipelas is amply corroborated by the work of Holman himself and also from the observations of Schottmueller,[18] Lorey,[19] Lyall,[20] Topley and Weir,[21] and others. Recently it has at least been suggested in the work of Howell,[22] and particularly by Tunnicliff,[23] that the particular form of Streptococcus pyogenes that occurs in erysipelas may present certain immunologic characteristics which would separate it from other varieties of Streptococcus pyogenes that occur in other diseases. This further differentiation, if true, is not of necessity consonant with the idea of specific localization that we have just mentioned and with the certainly erroneous idea of a common degree of pathogenicity between animal species. At all events, we may regard it as settled that Streptococcus pyogenes in its most recent sense is the cause of nearly every case of erypiseplas.

Further consideration of the experimental disease as produced in rabbits leads, we believe, to certain interesting conclusions on the nature of erysipelas, particularly in reference to the existence of transitory immunity and also as bearing on the more general problem of local, as opposed to a general, immunity.

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[12] Baumgartens Jahresbericht, 1886, 2, p. 15.

[13] Jour. Am. Med. Assn., 1915, 65, p. 1687.

[14] Deutsch. med. Wchnschr., 1886, No. 47.

[15] Thèse de Bordeaux, 1885.

[16] Virchows Archiv, 1887, 107, p. 209.

[17] Jour. Med. Research, 1916, 34, p. 377.

[18] München. med. Wchnschr., 1903, 50, p. 849.

[19] Ztschr. Hyg. u. Infektionskr., 1909, 63, p. 135.

[20] Jour. Med. Research, 1914, 30, p. 487.

[21] Jour. Path. and Bacteriol., 1921, 24, p. 333.

[22] Jour. Infect. Dis., 1918, 22, p. 230.

[23] Jcur. Am. Med. Assn., 1920, 75, p. 1339.

We have already referred to the original observations of Fehleisen,[4] who found that he could produce human erysipelas by means of pure cultures of his streptococcus in 6 out of 7 cases of malignant tumor in which he practiced inoculation. On reinjecting 2 of these 6 positive cases (cases 3 and 5) from 3 to 4 weeks subsequently, no erysipelas followed, and yet in the second of these cases the first inoculation had not only been positive, but had followed a spontaneous erysipelas which occurred about 10 months previously. These observations, limited as they were, certainly strongly suggested a transitory immunity and were so accepted by Fehleisen and later by Metchnikoff.[16] They were, however, not so regarded as indicative of active immunity by Koch and Petruschky[24] in the light of their own experiments. The first experimental study of this condition of streptococcus immunity was, we believe, made by Meierowitsch[25] in 1887, who found that healed erysipelas in rabbits protects against new inoculation for a period of from 1 to 2 months. Similar observations were made by Roger[26] a few years later, and he makes the definite statement that not only is the inoculated ear protected, but likewise the other ear. These studies were followed by similar work by Gromakowsky,[27] who further makes the interesting observation that rabbits that have become insusceptible to superficial skin inoculation do not always withstand a fatal intraperitoneal dose. Denys and Leclef[28] produced erysipelas with very small amounts of a broth culture of some of the strains they employed and found recovery was followed by little or no reaction on second inoculation. A subsequent piece of work by Cobbett and Melsome[29] is interesting in this connection. They failed to obtain erysipelas on reinoculation in the same ear during a period roughly of from 22 to 100 days following the first successful inoculation. They also found that there is some resistance when the second inoculation is given in the other ear, although it is not so marked as when given in the first ear. They interpreted this partial protection of the other ear as due to a condition of general immunity as against the purely local immunity present in the first ear. A second inoculation in the protected ear causes a transitory and limited infection, which is accompanied by an exaggerated leukocytic response. In the second article of Cobbett and Melsome[30] an attempt is made to estimate the factors concerned in this local immunity. Roger[31] had previously shown that successful experimental erysipelas depends rather closely on the amount of blood supplied to the part as influenced by experiments which affect the nervous control of the blood vessels. Cobbett and Melsome's work likewise indicates that a nonspecific inflammation such as that produced by mustard oil will prevent successful inoculation.

It is with these observations in mind that we have been interested in studying experimental streptococcus erysipelas more attentively. The question arises as to the actuality and duration of an acquired active immunity to this process, and further interesting relations have been suggested by this work as to the possible existence of a local immunity. The general question of a local immunity as contrasted with general immunity has been raised at intervals through the observations of Roemer,[32] von Dungern,[33] Wassermann,[34] Hektoen,[35] and more recently, Besredka.[36] It would take us too far afield to discuss these studies at this point, but at all events it may be said that the actual existence of a purely local immunity has never been satisfactorily proved.

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[24] Ztschr. f. Hyg. u Infektionskr., 1896, 23, pp. 267 and 474.

[25] Centralbl. f. Bakteriol., Ref., 1888, 3, p. 406.

[26] Compt. rend. Soc. de biol., 1890, 42, p. 573.

[27] Ann. de l'Inst. Pasteur, 1895, 9, p. 620.

[28] La Cellule, 1845, 11, p. 177.

[29] Jour. Path. & Bacteriol., 1896, 3, p. 39.

[30] Centralbl. f. allg. Path. u. path. Anat., 1898, 9, p. 827.

[31] Compt. rend Soc. de biol., 1890, 42, p. 646.

In previous articles we have discussed rather fully the form of experimental streptococcus empyema, which is produced regularly in rabbits by means of a strain of Streptococcus pyogenes ("H") originally obtained from a case of human empyema following measles and subsequently raised in virulence for rabbits by repeated passage through the pleural cavity of these animals. Although at first it produced a purulent and fatal empyema in rabbits only irregularly and in relatively large doses, the passage strain from the fourth generation on caused a characteristic and eventually fatal syndrome in every instance, even when very small amounts were used. One-tenth or 0.2 c c of a 24-hour broth culture of this organism, obtained immediately from the pleural fluid of a rabbit previously dead of the disease, is always fatal in from 4 to 5 days. Much smaller amounts are usually fatal, the minimal lethal dose apparently being 0.001 of a c c. The virulence of this passage strain is found not to fluctuate when we employ the pleural fluid from a fatal rabbit empyema as a standard source of culture. This pleural fluid contains living and virulent streptococci for several months when kept in the icebox, and has never in our experience become contaminated, in spite of repeated and often not overcareful opening of the tube for the inoculation of mediums. We do not, however, use any given pleural fluid for a period longer than one month. Although at first only 5% rabbit serum broth cultures were employed for inoculation, equally good results in the same doses have been obtained by using plain infusion broth of a p_H of from 7.4 to 7.8.

This empyema strain shows no distinct evidence of an elective affinity for the pleural cavity when administered intravenously. The passage pleural culture given by this route kills of a general septicemia with or without localized arthritis, but only in a minimal dose distinctly larger (0.01 to 0.1 c c) than by intrapleural inoculation. One-half of a cubic centimeter of bloody serous fluid was indeed found in the pleural cavity of one out of 19 animals that died after intravenous injection of various doses of pleural passage culture, and this fluid gave positive cultures of streptococcus. The exudate, however, showed little resemblance to the extensive purulofibrinous exudate which regularly follows the injection into the pleura. Several intraperitoneal injections have been accompanied by pleurisy, which can be explained on the basis of simple extension, as in the usual involvement of the pericardium following the intrapleural doses.

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[32] Arch. f. Opth., 1902, 54, p. 99.

[33] Die Antikörper, 1903.

[34] Ztschr. f. Hyg. u. Infektionskr., 1905, 50, p. 331.

[35] Jour. Infect. Dis., 1911, 9, p. 103.

[36] Ann. de l'Inst. Pasteur, 1919, 33, pp. 301 and 882; 1920, 34, p. 361; 1921, 35, p. 422.

When a small amount (0.1 c c) of the pleural passage culture of S. pyogenes "H" is injected intradermally near the midline on the back in any region from the scapula posteriad, a uniform erysipelas-like involvement of the regional lymphatics follows; this dose, however, is rarely fatal. With a larger dose (0.2 c c) the local reaction is also pronounced, and the animal dies of general septicemia. With smaller doses or with less virulent cultures, either only a slight reddening or a small pustule occurs. These observations correspond to the earlier investigations of the specificity of the erysipelas streptococcus by the observers to whom reference has been made. The original stock culture "H" from which our passage culture was derived and which has been conserved on rabbit blood agar for several years, produces no lesion intracutaneously with the regular doses. The results on which these statements are based are summarized in table 1.

TABLE 1

ERYSIPELAS PRODUCED BY PASSAGE "H" AND STOCK "H" CULTURES OF STREPTOCOCCUS FOLLOWING INTRADERMAL INJECTION IN VARIOUS PARTS OF THE BACK IN RABBITS

	Dose, C c	Total Number of Rabbits	Positive Lesion	Percentage	Death
Stock culture "H"...........	0.2	9	1	11.1	
Stock culture "H"...........	0.1	4	0	0	
Passage culture "H"........	0.2	8	8	100	7 (87.5%)
Passage culture "H"........	0.1	23	22	95.6	2 (9%)

In our experience inoculation on the back gives a much more definite and regular lesion than inoculation intracutaneously on the ear, where irregular and localized reactions were produced with 0.1 c c of our passage culture. A typical erysipelas involving the entire ear and lasting for several weeks follows the injection of 0.2 c c of the culture and is not followed by death, as when given on the back.

We are able then with regularity, or at least in over 95% of cases, to produce a characteristic erysipelatous lesion on the back of rabbits with 0.1 c c of the passage culture. Following the intracutaneous inoculation near the midline of the back, a red, hot, indurated area is evidenced at the end of 24 hours, which in 2 or 3 days extends to about one-half the distance toward the midline of the abdomen. It takes the form of an indurated ribbon measuring from 2 to 3 cm. in width, with sharply raised margins, which spreads down the lymphatics, but not laterally, so that by the third to the fifth day it has reached the midline of the abdomen and then spreads anteriorly for from 2 to 5 cm. The lesion then remains stationary for about 2 weeks, during which period it usually breaks down in the center at one or more points and on pressure exudes a few drops of pus, which on culture is found to contain the streptococcus. The lesion then gradually contracts, is covered with brownish scales and eventually heals in from 3 to 4 weeks with no marked cicatrization and an almost complete return to normal condition. We have found in the few cases studied that the temperature rises from 1 to 3 degrees for the first 2 days following the intradermal injection. This fever, which is intermittent, is accompanied by a moderate increase of the polymorphonuclear leukocytes.

As already stated, when the larger dose of 0.2 c c of a 24-hour culture instead of 0.1 c c was employed, 7 of 8 animals so inoculated died in periods of from 24 hours to 20 days. In 2 animals only out of 23 has a fatal result followed the smaller dose. We have then in 0.1 c c of a 24-hour infusion broth passage culture a symptomatic, but rarely fatal, dose and in 0.2 c c a fatal dose. At this point a comparison of the minimal lethal dose by the different routes we have employed, intradermal, intravenous and intrapleural, is of interest as bearing again on the possible elective specificity of the passage pleural strain.

TABLE 2

MINIMAL LETHAL DOSE OF STREPTOCOCCUS PYOGENES "H," BOTH "STOCK" AND "PLEURAL PASSAGE" CULTURES FOR RABBITS BY DIFFERENT ROUTES

Route	Stock "H" Cc	Passage "H" Cc
Intradermal	Negative to 0.2	0.2
Intravenous	Negative to 10	0.1
Intrapleural	0.1	0.001

Certain interesting conclusions appear from inspection of table 2:

In the first place, the strain of streptococcus "H," both stock and passage, is very much more pathogenic when administered by the pleura than by either the intradermal or the intravenous route; and second, it has been raised in virulence markedly by passage through the pleura, but so far as can be observed, there is no selective increase in pathogenicity, that is to say, the pleural passage strain is relatively no more pathogenic by the pleural route than by either of the other 2 routes. The stock culture was not tested by the intradermal or intravenous route in doses larger than those that have been indicated, and it should be stated that the dose of 0.2 c c of stock culture intradermally not only is not fatal, but does not produce the characteristic erysipelas.

It is of interest here to recall the variations that have so far been observed between the original "stock" and "passage" cultures of the strain of the streptococcus with which we have been working in this laboratory for the past several years. The passage culture differs from the stock in the following particulars:

1. It is more pathogenic for rabbits, irrespective of route, as outlined in the foregoing.

2. It has an increased ability to produce hemotoxin (Cook, Mix and Culvyhouse [4]).

3. It shows a marked difference in nitrogen metabolism in its early growth in certain mediums. This is indicated in the passage strain by a decrease in amino acid output, coupled with increased ammonia excretion, while the stock strain shows the opposite set of conditions (Foster [7]).

IMMUNITY FROM EXPERIMENTAL ERYSIPELAS

Rabbits that have recovered from the intradermal symptomatic dose of the passage culture, that is to say, from experimental erysipelas, have been tested at different intervals for their protection against subsequent inoculation, and the results are briefly recorded in table 3.

TABLE 3

PROTECTION AGAINST ERYSIPELAS AFFORDED BY ONE OR MORE INJECTIONS OF A SYMPTOMATIC DOSE OF PASSAGE CULTURE INTRADERMALLY

Number of Animals	Days After First Inoculation Tested	Protected	
		Number	Percentage
3	7- 14	0	0
19	20-141	17	89.4

It appears that a definite immunity is produced by recovery from experimental erysipelas as tested 3 or more weeks following the primary inoculation. The 2 unprotected animals were tested the second time 60 and 141 days after the primary inoculation, which suggests that the immunity acquired is temporary, although, as the table shows, one animal at least was protected for 3½ months. A word should be said as to what constitutes our criterion of protection against this experimental lesion as we have described it. It was found after a number of measurements that the average distance from the vertebrae to the midabdominal line in rabbits such as we have employed in these experiments, that is to say, from 2,000 to 3,500 gm. in weight, is on an average 16 cm. An extension of the lesion to this point has been designated as a triple plus or complete reaction (+ + +); extension for one-half the distance, i. e., about 8 cm., a double plus reaction (+ +), and anything less than this distance is designated as a single plus reaction (+). In all but one of the 23 control animals, the inoculation extended to a + + degree, and in most instances the entire distance (+ + +), actually in 15 animals of 23. We have, therefore, adopted a double plus reaction, that is, extension one-half way to the midline, as evidence of a failure of protection, and the figures indicated in table 3 are based on this criterion. A temporary local induration lasting for 2 or 3 days and approaching a + reaction may be obtained with stock "H" culture or other nonpathogenic cultures of streptococcus or even with streptococcus vaccines.

The animals that were tested for protection have in all instances received the second test inoculation at a different point from the one utilized in the first inoculation, that is to say, when the first positive lesion was obtained on the right side of the body, the second inoculation was given to the left of the vertebral column. It would not seem, then, as might be indicated from the work of earlier investigators, particularly Cobbett and Melsome,[29,30] that protection is present only in a locally immunized or simply an inflamed area. Furthermore, inoculation over the scapular region protects perfectly well against second inoculation several inches posteriad on the other side. Primary inoculations of 0.1 c c in the ear which do not produce a well-defined lesion, as we have already mentioned, do not protect, but a dose of 0.2 c c in that locality produces a marked lesion without death and gives perfect protection against a second inoculation on the back. The degree of primary reaction then is of importance in determining protection. This is concretely evidenced from our results on reinoculation of a symptomatic dose in a number of animals which on primary intradermal injection of this dose into the back had shown no characteristic lesion, owing to previous treatment by serum or vaccine. Six out of 8 such animals, when tested between the 26th and 76th day following the initial dose of passage culture, showed no protection against erysipelas. The fact that such animals had not reacted to the first symptomatic dose of course shows some degree of protection from the vaccines themselves and logically introduces the question as to whether animals can be protected in any other way than by recovery from erysipelas.

The next point to consider is the protection that may be afforded by stock cultures which produce small lesions or none at all; by subsymptomatic doses of the passage culture; and by vaccines prepared from the passage culture. In tables 4 to 7 are summarized the results of these experiments.

The data embodied in these tables will be understood with the following brief statement:

(1) Living Cultures of Stock Streptococcus "H": These may be inoculated repeatedly in doses at least as large as 0.2 c c of a 24-hour injection broth culture intradermally without producing more than a slight local induration. The number of injections and the amounts employed are indicated in the table.

(2) Saline Passage "H" Vaccines: Infusion broth was inoculated with passage streptococcus "H," incubated for 48 hours, centrifugalized and the sediment suspended in absolute alcohol, shaken, recentrifugalized, suspended in ether and dried over $CaCl_2$ to constant weight. This dried culture was then ground in an agate mortar [2] and suspended in 0.5% phenolated saline solution in such volume that 1 c c contained 10 c c of the original culture fluid.

(3) Oil Passage "H" Vaccines: Killed and dried by alcohol and ether parallel to the saline vaccines, but suspended in 0.5% phenolated cotton seed or olive oil.

The following conclusions may be drawn from the experimental evidence presented in tables 4 to 7.

It is evident that 0.1 c c of the passage culture is about the smallest amount that will produce a characteristic erysipelas, as we have defined and limited it. At least, 0.01 c c and less fails to do so. A barely subsymptomatic dose of living culture, 0.01 c c, may produce protection (rabbit 553), but a smaller dose 0.001 c c, does not.

It is clear from rabbit 550 and table 6 that ordinary heat killed or alcohol killed vaccine, whether sensitized or plain, does not protect even when given repeatedly. Oil vaccines prepared in the same general way (table 7) do, however, protect in the majority of cases.

Living stock cultures of "H," which in itself produces no lesion, will, when given repeatedly, protect against the passage culture. Apparently one or two injections do not usually suffice to protect, as when a lesion is produced with a passage culture.

TABLE 4

Results in Four Rabbits Given Different Preparations of Passage Streptococcus "H" and Following an Interval of 28 Days Tested with a Symptomatic Dose of Passage Culture Streptococcus "H," 0.1 C c, for Protection Against Erysipelas

Number	First Inoculation	First Lesion	Protection on Second Inoculation
558	0.1 c c Passage "H"........................	++	Nearly complete
553	0.01 c c Passage "H".......................	±	Complete
551	0.001 c c Passage "H"......................	+	None
550	0.1 c c Passage "H" 65 C....................	0	None

TABLE 5

Results in Rabbits Given Various Amounts and Number of Doses of Living Stock Culture "H" and Tested Subsequently with the Symptomatic Dose of Passage Culture

Rabbit	Route	Number Injections	Billions	Duration	Lesions	Protection
760	Intradermal	5 living stock "H"	0.5	55	0	+
823	Intradermal	4 living stock "H"	0.004	30	0	+
646	Intradermal	3 living stock "H"	0.6	21	+	+
765	Intradermal	3 living stock "H"	0.3	39	0	+
562	Intradermal	2 living stock "H"	0.2	31	+	+
742	Intradermal	2 living stock "H"	0.2	31	+++	0
552	Intradermal	1 living stock "H"	0.1	28	+++	0
573	Intradermal	1 living stock "H"	0.2	28	++	Receding ±*
574	Intradermal	1 living stock "H"	0.2	28	+++	0
575	Intradermal	1 living stock "H"	0.2	28	+++	0
576	Intradermal	1 living stock "H"	0.2	28	0	+

* In 2 instances in incompletely immunized animals, a lesion progressed to ++ in 3 or 4 days and then receded. This never occurs in the normal animal, and we regard it as evidence of partial protection.

TABLE 6

Results in Rabbits Given Various Amounts and Varying Number of Injections of Killed Vaccines, Sensitized and Unsensitized, and Subsequently Tested for Protection Against Erysipelas

Rabbit	Route	Number Injections	Billions	Duration	Lesions	Protection
626	Intradermal	4 sensitized vaccine	2	22	+++	0
625	Intradermal	4 sensitized vaccine	2	22	+++	0
891	Intradermal	5 plain vaccine	2.5	26	+++	0
657	Intradermal	3 plain vaccine	1.5	29	+++	0
660	Intradermal	3 plain vaccine	1.5	29	+++	0

TABLE 7

Results in Rabbits Given Various Amounts and a Varying Number of Injections of Killed Oil Vaccines and Tested Subsequently for Protection Against Erysipelas

Rabbit	Route	Number Injections	Billions	Duration	Lesions	Protection
656	Intradermal	3 oil vaccine	1.5	29	0	+
524	Intradermal	3 oil vaccine	3	28	+	+
688	Intradermal	3 oil vaccine	3	28	+++	0
628	Intradermal	3 oil vaccine	1.5	21	0	+
663	Intradermal	2 oil vaccine	1	22	+++	0

The failure of ordinary vaccine to protect against erysipelas is consonant with other results that we have obtained in immunizing against intravenous or intrapleural infection with the streptococcus; namely, that living cultures produce a much more marked grade of active immunity than do killed cultures. It is evident in the immunization against erysipelas that oil vaccine is less rapidly absorbed from local areas than a saline suspension. This fact, among others, suggests that this particular immunity is to some extent at least a local phenomenon and suggests other methods of comparison to settle these points. It has appeared to us for some time in the course of these investigations on the different routes of immunization and infection with the streptococcus that local phenomena are important; for instance, we have apparently been able to immunize against pleural infection when vaccines followed by small amounts of living stock cultures have been given into the pleura, but not when the same amounts were injected intravenously or intradermally. We are not as yet able to present wholly convincing evidence of the superiority of local intrapleural immunization, owing to a failure in the majority of our early experiments to obviate the marked differences in the infecting dose by the different routes. The test doses of our passage culture are practically the same by intravenous and intradermal routes, that is to say, 0.1 c c intradermally gives erysipelas without death, and intravenously is fatal.

In table 8 are displayed the histories of 8 rabbits that were immunized by intravenous injections of killed streptococcus vaccines, followed by living stock or passage cultures of streptococcus "H." They are all animals which would usually have been found to be unaffected by a test dose (0.1 or 0.2 c c) of passage culture administered intravenously. They would moreover all, except perhaps animal 120, have been protected against erysipelas if the immunizing injections had been given intradermally, and yet when tested by intradermal injections of 0.1 c c of passage "H," 5 of the 8 (62.5%) developed characteristic lesions.

In table 9 are listed 11 animals which were immunized by the intradermal route and then actually proved to be protected against erysipelas. They were then injected intravenously with the same dose of the same passage culture and only 7 of the animals survived. Here the chances were against demonstration of a difference in protection between intradermal and intravenous routes, because the test intradermal dose of course increased the immunization. Four other animals that were not given the intradermal test, but that by comparison were protected against erysipelas, all died on intravenous injection.

Rabbits that have recovered from several carefully graded intrapleural injections, first of vaccine and then small amounts of living stock culture, are found to be protected not simply against multiple fatal doses intrapleurally, but against intradermal erysipelas as well.

All these experiments we advance simply as indicative that there really exists a local immunity as contrasted with a general immunity. Complete demonstration of this fact must await extensive comparative series of animals immunized by the various routes and subsequently tested simultaneously by each of these routes. The difficulties of this demonstration are not simply in the number of animals required, but in carefully equilibrating the multiple of lethal doses employed in accordance with the route utilized. Furthermore, to demonstrate local immunity, precisely the right degree of protection must be attained and no more, or the local immunity will pass over into a general form.

TABLE 8

PROTECTION AGAINST EXPERIMENTAL ERYSIPELAS AFFORDED BY INTRAVENOUS IMMUNIZATION; 0.1 C C OF PASSAGE "H" CULTURE GIVEN INTRADERMALLY AS A TEST

Rabbit	Number and Kind of Injections	Dose in Billions	Duration, Days	Lesions	Protection
120	14 dead saline vaccine	14	100	+++	0
506	3 oil vaccine	7.5	38	0	+
611	2 killed sensitized vaccine	7.5			
	3 living stock "H"	+0.0003 c c	41	+++	0
612	Idem	Idem	41	+++	Death 0
697	3 oil vaccine	7.5			
	1 living passage "H"	+0.2	60	0	+
818	4 living stock "H"	0.004	20	++	0
832	1 living passage "H"	0.01	97	0	+
527	1 living passage "H"	0.2	37	+++	0

TABLE 9

PROTECTION OF RABBITS AGAINST A FINAL INTRAVENOUS INJECTION OF 0.1 C C OF PASSAGE "H" *

Rabbit	Number of Injections	Dose in Billions	Duration	Result
763	6	0.6	12 weeks	Recovered
622	1	0.1	3 weeks	Recovered
663	4	2.2	8 weeks	Recovered
837	5	0.5	19 weeks	Dead, 14 days
823	5	0.5	7 weeks	Recovered
822	4	0.4	4 weeks	Dead, 3 days
641	9	0.9	10 weeks	Recovered
742	8	0.8	15 weeks	Recovered
660	5	0.2	8 weeks	Recovered
646	5	0.9	11 weeks	Dead, 6 days
833	5	0.5	19 weeks	Dead, 11 days

* Living stock cultures were used to immunize and perfect protection against erysipelas was assured by intradermal injection of 0.1 c c of passage culture before the intravenous test was given.

CONCLUSIONS

The passage pleural culture of Streptococcus pyogenes ("H"), with which we have been working for several years, has acquired new and relatively constant properties. In addition to certain changes in its metabolic functions, it has gained a marked pathogenicity for rabbits, whereas the original stock culture has little. Constant empyema is produced by direct inoculation of very small amounts of the passage strain. In considerably large doses (about 100 times) it will usually produce a fatal septicemia when given intravenously and a recoverable erysipelas when injected intradermally in the back. Somewhat larger doses are necessary to produce erysipelas in the ear. This constant lesion is not produced by the stock culture.

Recovery from erysipelas from a period roughly of 3 weeks after inoculation confers complete protection against intradermal reinoculation, irrespective of its locality. This protection usually lasts for at least 3 months. Repeated injections of heat or alcohol killed vaccines from the passage strain do not protect against the local lesion. Oil vaccines in the same amount frequently protect. Several injections of the original living stock culture, which produces no lesion, protect against the passage strain.

The fatal intravenous dose and the symptomatic intradermal dose of the passage culture are practically the same. Complete protection against intradermal inoculation by previous intradermal infection or immunization frequently does not protect against intravenous infection. The reverse set of conditions apparently also prevails; that is, intravenous immunization protects better against intravenous than against intradermal infection.

These last facts, together with other indications from our own experiments and from observations of others, point strongly to the existence of a true local tissue immunity following local streptococcus infection or immunization.